Octreotide Inhibits the Bilirubin Carriers Organic Anion Transporting Polypeptides 1B1 and 1B3 and the Multidrug Resistance-Associated Protein 2

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Abstract: The somatostatin analog octreotide can lead to hyperbilirubinemia without evidence of liver injury. Here we investigate whether octreotide inhibits the main sinusoidal/canalicular bilirubin carriers and whether it is a transport substrate. Octreotide showed the most potent inhibitory effect toward OATP1B1-mediated transport and weaker inhibition for OATP1B3- and MRP2-mediated transport. Octreotide had no effect on OATP2B1-mediated transport. Octreotide inhibited [(3)H]estradiol-17- -glucuronide (E17 G) influx mediated by OATP1B1, 1B3, and multidrug resistance-associated protein 2 (MRP2) in a concentration-dependent manner, and the IC50 values were computed to be 23 M (95% confidence interval [CI] 18-29), 68 M (95% CI 50-91), and 116.6 M (95% CI 74.5-182.4), respectively. The interaction between octreotide and OATP1B1 was further studied. Inhibition of [(3)H]E17 G OATP1B1-mediated transport was purely competitive with no changes in maximum transport capacity (Vmax) and a twofold Km increase when the influx kinetics of [(3)H]E17 G were measured in the presence of octreotide (8.8 ± 3.1 versus 4.4 ± 1.2 M, P = 0.03). The inhibition constant (Ki) of octreotide for the transport of [(3)H]E17 G was calculated at 33.5 ± 5.5 M. Uptake of radiolabeled octreotide by OATP1B1-CHO cells was higher than in wild-type CHO cells and nonlabeled octreotide at the extracellular compartment was able to trans-stimulate the OATP1B1-mediated efflux of intracellular [(3)H]E17 G, suggesting that octreotide is a substrate of OATP1B1. In summary, this study shows interaction of octreotide on the human hepatocellular bilirubin transporters OATP1B1, OATP1B3, and MRP2, notably OATP1B1. These findings are in line with the clinical observation that a fraction of patients under treatment with octreotide exhibit hyperbilirubinemia.

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Octreotide Inhibits the Bilirubin Carriers Organic Anion Transporting Polypeptides 1B1 and 1B3 and the Multidrug Resistance-Associated Protein 2

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ABSTRACT

The somatostatin analog octreotide can lead to hyperbilirubinemia without evidence of liver injury. Here we investigate whether octreotide inhibits the main sinusoidal/canalicular bilirubin carriers and whether it is a transport substrate. Octreotide showed the most potent inhibitory effect toward OATP1B1-mediated transport and weaker inhibition for OATP1B3- and MRP2-mediated transport. Octreotide had no effect on OATP2B1-mediated transport. Octreotide inhibited [^{3}H]estradiol-17-β-glucuronide (E17βG) influx mediated by OATP1B1, 1B3, and multidrug resistance-associated protein 2 (MRP2) in a concentration-dependent manner, and the IC_{50} values were computed to be 23 μM (95% confidence interval [CI] 18-29), 68 μM (95% CI 50-91), and 116.6 μM (95% CI 74.5–182.4), respectively. The interaction between octreotide and OATP1B1 was further studied. Inhibition of [^{3}H]E17βG OATP1B1-mediated transport was purely competitive with no changes in maximum transport capacity (V_{max}) and a twofold K_m increase when the influx kinetics of [^{3}H]E17βG were measured in the presence of octreotide (8.8 $\pm$ 3.1 versus 4.4 $\pm$ 1.2 μM, $P = 0.03$). The inhibition constant (K_i) of octreotide for the transport of [^{3}H]E17βG was calculated at 33.5 $\pm$ 5.5 μM. Uptake of radiolabeled octreotide by OATP1B1-CHO cells was higher than in wild-type CHO cells and nonlabeled octreotide at the extracellular compartment was able to *trans*-stimulate the OATP1B1-mediated efflux of intracellular [^{3}H]E17βG, suggesting that octreotide is a substrate of OATP1B1. In summary, this study shows interaction of octreotide on the human hepatocellular bilirubin transporters OATP1B1, OATP1B3, and MRP2, notably OATP1B1. These findings are in line with the clinical observation that a fraction of patients under treatment with octreotide exhibit hyperbilirubinemia.

Introduction

Somatostatin is an endogenous inhibitory peptide (14 amino acids) that modulates neurogastroenterological motility at various levels. By binding specific membrane receptors, somatostatin inhibits the secretion of neurotransmitters, growth hormone, thyroid-stimulating hormone, pancreatic enzymes, and neuropeptides (Theodoropoulou and Stalla, 2013).

Because of its broad and potent antisecretory effect, somatostatin would have many pharmacological applications; however, its short half-life (~3 minutes) and the requirement for intravenous administration make it difficult to use in clinical practice (Patel and Wheatley, 1983; Lamberts et al., 1996). The recognized therapeutic benefits of somatostatin led to the development of somatostatin analogs with an improved pharmacological profile. The first to be introduced for clinical use was octreotide, a cyclic octapeptide with a longer half-life (~2 hours) and greater inhibitory effect compared with that of the endogenous somatostatin. Octreotide is used in the treatment of many hyperfunctional organ conditions such as acromegaly, thyroid-stimulating hormone-secreting pituitary adenomas, and neuroendocrine tumors (Lamberts et al., 1996; Theodoropoulou and Stalla, 2013). Octreotide is also employed as a radioligand for the imaging and treatment of somatostatin receptor-expressing tumors (i.e., neuroendocrine tumors) (Toumpanakis and Caplin, 2013). Octreotide is mainly cleared into bile in unchanged form (Berelowitz et al., 1978; Bauer et al., 1982; Lemaire et al., 1989) and can lead to hyperbilirubinemia and asymptomatic gallbladder stone formation, albeit without signs of liver injury (Arosio et al., 1988; Hussaini et al., 1994; Radetti et al., 2000; Crook and Steger, 2001; Pereira et al., 2001; Koren et al., 2013). Nevertheless, the mechanism of the vectorial transport of octreotide from portal blood into bile has been only partially studied with conflicting results and none has investigated the interaction of octreotide with the main human liver transporters (Terasaki et al., 1995; Yamada et al., 1996, 1997; Gutmann et al., 2000).

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ABBREVIATIONS: ASP+, 4-(4-(dimethylamino)-styryl)-*N*-methylpyridinium iodide; BSP, sulphobromophtalein; CI, confidence interval; E17βG, estradiol-17-β-glucoronide; EHBR, Eisai hyperbilirubinemic rats; E3S, estrone-3-sulfate; ICG, indocyanine green; NS, not significant; NTCP, Na^+-taurocholate cotransporting polypeptide; OATP, organic anion transporting polypeptide; OCT, organic cation transporter; WT, wild-type.

The objective of the current study was a comprehensive analysis of the potential role of the main sinusoidal/canalicular transport systems in the clearance of octreotide by using in vitro models that selectively expressed the different human hepatic membrane transporters.

Methods

Reagents. [6,7-^{3}H(*N*)]estrone-3-sulfate ([^{3}H]E3S, 45.6 Ci/mmol), [6,7-^{3}H(*N*)]estradiol-17-β-glucuronide (E17βG, 45 Ci/mmol), [^{3}H(G)] taurocholic acid (5.0 Ci/mmol) were purchased from PerkinElmer (Boston, MA). The generally labeled [^{3}H]octreotide was synthesized by Moravek Biochemicals (Brea, CA). Nonlabeled octreotide was purchased from Abbiotec (San Diego, CA). Nonlabeled E3S, taurocholic acid sodium salt, E17βG, indocyanine green (ICG), and sulphobromophtalein (BSP) were provided from Sigma-Aldrich (St. Louis, MO) and 4-[4-(dimethylamino)-styryl)-*N*-methylpyridinium iodide] (ASP+) by Molecular Probes/Life Technologies (Carlsbad, CA).

Cell Lines. Wild-type (WT) CHO and HEK293 cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (Gibco, Paisley, UK), 100 units/ml penicillin, and 100 mg/ml streptomycin at 37°C in a humidified atmosphere with 5% CO_2. Wild-type CHO were further supplemented with 0.05 mg/ml L-proline.

OATP1B1 (SLCO1B1), OATP1B3 (SLCO1B3), and OATP2B1 (SLCO2B1) stably transfected CHO cells (Treiber et al., 2007; Gui et al., 2008) were grown under selective pressure with Geneticin G-418 (500 μg/ml) (Gibco). CHO FlpIn cells stably expressing the Na^+-taurocholate cotransporting polypeptide (NTCP-SLC10A1) were grown in Ham's F-12 medium containing 10% fetal bovine serum, 100 units/ml penicillin, 100 mg/ml streptomycin, 1 mM L-glutamine, and 500 μg/ml hygromycin B as selecting agent (de Waart et al., 2010). HEK293 cells stably transfected with the human organic cation transporter (OCT1-SLC22A1), kindly provided by Dr. Hermann Koepsell, Würzburg, Germany, were supplemented with Geneticin G-418 (600 μg/ml) (Thevenod et al., 2013).

Sf21 insect cells were grown in a humidified atmosphere at 27°C on 10-cm Petri dishes in BD BaculoGold TNM-FH insect medium (BD Bioscience, Allschwil, Switzerland) supplemented with 100 units/ml penicillin and 100 mg/ml streptomycin.

MRP2 Expression in Insect Cells. Sf21 cells were infected with baculovirus human MRP2 [multidrug resistance-associated protein 2 (MRP2); ATP-binding cassette C2] (de Waart et al., 2006; Guyot et al., 2014). The isolation of membrane vesicles from infected cells was performed as previously described (Gerloff et al., 1998). The vesicles were resuspended in 50 mM sucrose, 100 mM KNO_3, 20 mM Hepes/Tris pH 7.4, and loaded with 1 mM cholesterol for optimal MRP2 transport activity (Guyot et al., 2014); a portion was used for protein determination by the bicinchoninic acid assay (Interchim, Montluçon Cedex, France), and the remaining part was used for the transport assay.

Transport Studies in Intact Cells. Initial uptake of tritiated or fluorescent compounds was measured using a protocol designed for rapid uptake determination in cells (Schroeder et al., 1998). Cells were seeded in 35-mm dishes at a density of 2.5×10^5 cells/dish and grown for 3 days in regular medium. For the seeding of WT-HEK293 and OCT1-HEK293 cells, 35-mm dishes were coated with 0.1 mg/ml poly-D-lysine (Sigma-Aldrich). Twenty-four hours before the uptake measurement, cells were treated with 5 mM sodium butyrate (Sigma-Aldrich) to induce gene expression (Kim and Lee, 2000–2001). Medium was aspirated and cells were rinsed twice with prewarmed transport buffer (116.4 mM NaCl, 5.3 mM KCl, 1 mM NaH_2PO_4, 0.8 mM $MgSO_4$, 5.5 mM D-glucose, and 20 mM Hepes/Tris, pH 7.4). When the transport of [^{3}H]octreotide was measured, 20 mM $MgCl_2$ was added to the transport buffer to reduce the nonspecific background signal, attributable presumably to the electrostatic binding of octreotide to cell membranes (Beschiaschvili and Seelig, 1991; Ben-Tal et al., 1997). The transport experiments in HEK293 cells were performed in a transport buffer containing 136 mM NaCl, 5.3 mM KCl, 1.1 mM KH_2PO_4, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, 11 mM D-glucose, and 10 mM Hepes/Tris, pH 7.4.

Cells were equilibrated in transport buffer at 37°C, and then the buffer was aspirated and transport buffer containing the labeled reagents was added. Uptake was stopped by quick aspiration of the labeled cocktail followed by extensive washing with ice-cold transport buffer. Cells were digested with 1 ml of 1% (v/v) Triton X-100. Lysate (500 μl) was mixed with 10 ml of Scintillation Liquid (Ultima Gold, PerkinElmer) and assessed for intracellular radioactivity by liquid scintillation counting. To measure the intracellular ASP+, the fluorescence (λ_{ex} = 485 nm, λ_{em} = 590 nm) of a 150-μl aliquot was measured on the Twinkle LB970 microplate fluorometer (Berthold Technologies, Bad Wildbad, Germany). Protein content was determined by the bicinchoninic acid protein assay on a 25-μl aliquot. In OATP/OCT1 transport experiments, OATP/OCT1-independent uptake was determined in WT-CHO and WT-HEK293 cells, respectively, and subtracted from total uptake to quantify OATP- and OCT1-mediated uptake.

When NTCP-mediated transport was measured, nonspecific transport was assayed in a Na^+-free buffer that maintained osmolarity by replacement of Na^+ with choline (116.4 mM choline chloride, 5.3 mM KCl, 1 mM KH_2PO_4, 0.8 mM $MgSO_4$, 5.5 mM D-glucose, and 20 mM Hepes/Tris, pH 7.4) (de Waart et al., 2010). Influx is expressed as picomoles of substrate per milligram of protein per minute.

For efflux studies, cells were incubated with 10 μM [^{3}H]E17βG for 30 minutes at pH 7.4, washed extensively in ice-cold drug-free buffer, and suspended into a large volume of prewarmed transport buffer at pH 7.4. At different time points, portions were injected into 0°C transport buffer and washed twice, and protein and radioactivity were analyzed as indicated above.

Membrane Vesicle Transport Studies. ATP-dependent uptake by MRP2 was measured using the rapid filtration technique as previously described (Gerloff et al., 1998). Briefly, vesicles were extracted and resuspended to a final concentration of 3.5 μg/μl in 50 mM sucrose, 100 mM KNO_3, 10 mM Hepes, adjusted with Tris to pH 7.4; 70 μg of vesicles were preincubated in a 37°C water bath, and uptake was initiated by injecting the radiolabeled compound dissolved in the uptake buffer (50 mM sucrose, 100 mM KNO_3, 12.5 mM Mg $(NO_3)_2$, 5 mM ATP, 10 mM Hepes/Tris, pH 7.4). Uptake was also measured in the absence of ATP, and ATP-dependent uptake was calculated as the difference in uptake in the presence and in the absence of ATP. Transport was stopped by adding 3 ml of ice-cold stop solution (50 mM sucrose, 100 mM KCl, 10 mM Tris/HCl, pH 7.4) followed by filtration through 0.45-μm nitrocellulose acetate filter (Sartorius, Göttingen, Germany). Afterward, the filter was extensively washed, dissolved in 3 ml of liquid scintillation fluid (Filter Count; PerkinElmer), and radioactivity was measured on a liquid scintillation counter. Influx is expressed as picomoles of substrate per milligram of protein per minute.

Statistical Analysis. Statistical comparisons were performed from three independent measurements, at least, with the two-tailed Student's *t* test using GraphPad Prism (version 5.0 for Windows, GraphPad Software, La Jolla, CA). The IC_{50} values were computed from a log (inhibitor) versus response curve derived from the formula: $Y = 100/[1+10^{(X-LogIC_{50})}]$, where X is the logarithm of the concentration of the inhibitor and Y is the response expressed as percentage of the control (no inhibitor).

Results

Effect of Octreotide on OCT1-Mediated Transport. OCT1 is the main cation transporter expressed at the basolateral membrane of hepatocytes. To determine whether octreotide (z = +2) interacts with this carrier, OCT1-HEK293 cells were coincubated for 2 minutes (linear range)

with 2 μM fluorescent substrate ASP+ and increasing concentrations of octreotide (Schlatter et al., 2002). Figure 1 illustrates that intracellular ASP+ decreased as the extracellular concentration of octreotide increased. The IC_{50} was computed to be 93.7 ± 1.1 μM [95% confidence interval (CI) 71.0–123.5].

Impact of Octreotide on the Transport Mediated by OATP1B1, 1B3, and 2B1. To assess the potential interaction of octreotide with OATP1B1 and 1B3, the influx of 1 μM [^{3}H]E17βG was assessed as a function of the extracellular concentration of nonlabeled octreotide. Because [^{3}H]E17βG is not a substrate of OATP2B1 (Tamai et al., 2001), 1 μM [^{3}H]E3S was used to determine the effect of nonlabeled octreotide on transport mediated by OATP2B1. Transport was performed over 1 minute, because over this time period uptake was unidirectional (unpublished data). The influx of [^{3}H]E17βG mediated by OATP1B1 (Fig. 2A) or OATP1B3 (Fig. 2B) was inhibited by nonlabeled octreotide in a concentration-dependent manner, and the IC_{50} values were computed to be 24.1± 1.1 μM (95% CI 19.2–30.4) and 72.7±1.2 μM (95% CI 47.9–110.4), respectively. Figure 2C shows that extracellular octreotide had no effect on the influx of [^{3}H]E3S mediated by OATP2B1. In the presence of 200 μM extracellular octreotide the influx of 1 μM [^{3}H]E3S was comparable to that in the octreotide-free buffer [11.0 ± 4.5 versus 10.6 ± 4.3 pmol/mg of protein/min, P = not significant (NS)]. As expected, ICG (5 μM) or BSP (5 μM), two potent inhibitors of OATP2B1-mediated transport (Kullak-Ublick et al., 2001; de Graaf et al., 2011), markedly reduced [^{3}H]E3S influx, by ~97% (0.3 ± 1.4 versus 10.6 ± 4.3 pmol/mg of protein/min, P = 0.02) and 75% (2.6 ± 0.6 versus 10.6 ± 4.3 pmol/mg of protein/min, P = 0.03), respectively, compared with uptake in the absence of inhibitors.

Impact of Octreotide on Na^+-Dependent Bile Salt Transport. In rat hepatocytes, octreotide has been suggested to be transported by the Na^+-dependent bile salt carrier (Terasaki et al., 1995). To investigate whether the human Na^+-taurocholate cotransporting polypeptide (NTCP) was inhibited by octreotide, the influx of 0.5 μM [^{3}H]taurocholic acid was assessed in NTCP-CHO cells after coincubation with nonlabeled octreotide. As a control, the influx of [^{3}H]taurocholic acid was also measured in the presence of ICG or BSP, known inhibitors of NTCP-mediated transport (Hagenbuch and Meier, 1994; de Graaf et al., 2011). Figure 3 shows that extracellular ICG (10 μM) or BSP (200 μM) markedly reduced

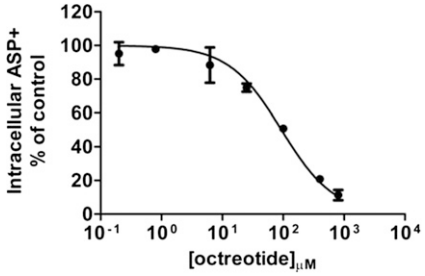

Fig. 1. Inhibitory effect of octreotide on OCT1-mediated transport. Influx of 2 μM fluorescent substrate ASP+ was assessed in OCT1-stably transfected HEK293 cells at pH 7.4 over 2 minutes in the absence (control) or presence of nonlabeled octreotide at the indicated concentrations. The uptake in wild-type HEK293 cells was subtracted from that in transfected cells to define the OCT1-specific transport. Data represent the mean ± S.D. from three independent experiments and are expressed as percentage of the control. The IC_{50} value was computed from a log (inhibitor) versus response curve.

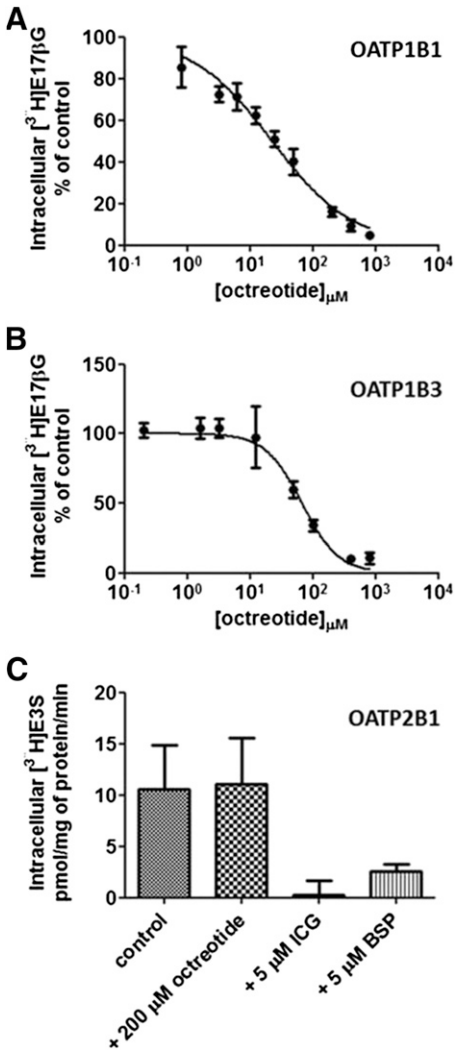

Fig. 2. Effect of octreotide on OATP-mediated transport. (A and B) Influx of 1 μM [^{3}H]E17βG was assessed in OATP1B1 and OATP1B3 stably transfected CHO cells at pH 7.4 over 1 minute in the absence (control) or presence of nonlabeled octreotide at the indicated concentrations. The data are expressed as percentage of the control, and the IC_{50} values were computed from a log(inhibitor) versus response curve. (C) Influx of 1 μM [^{3}H]E3S was assessed in OATP2B1-transfected CHO cells at pH 7.4 over 1 minute in the absence (control) or presence of nonlabeled octreotide, ICG, or BSP at the indicated concentrations. For all the experiments, the uptake in wild-type CHO cells was subtracted from that in transfected cells to define the OATP-specific transport. Data represent the mean ± S.D. from three independent experiments, at least.

[^{3}H]taurocholic acid influx by ~65 and 95%, respectively, compared with uptake in the absence of inhibitors. When the influx of [^{3}H]taurocholic acid was measured in the presence of 200 μM octreotide in the extracellular compartment, there was no difference in the amount of intracellular [^{3}H]taurocholic acid compared with that in the absence of octreotide (10.5 versus 11.2 pmol/mg of protein/min, P = NS).

Impact of Octreotide on the Transport of [^{3}H]E17βG Mediated by MRP2. Organic anion transporting polypeptides (OATPs) share a variety of substrates with the canalicular efflux pump MRP2 (Suzuki and Sugiyama, 1999). The interaction of octreotide with MRP2 was evaluated by measuring the transport of radiolabeled E17βG in Sf21 membrane vesicles. Transport was measured over 90 seconds, because uptake slopes are linear over this period and extrapolate

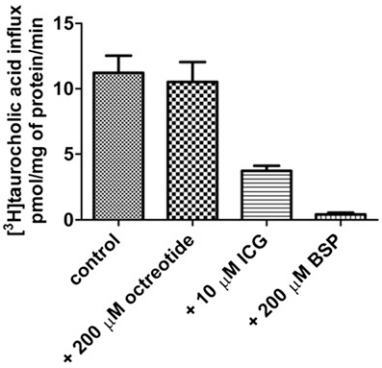

Fig. 3. Effect of octreotide and other compounds on NTCP-mediated [^{3}H]taurocholic acid influx. NTCP-CHO cells were incubated for 1 minute at pH 7.4 with 0.5 μM [^{3}H]taurocholic acid in the presence or absence of nonlabeled octreotide, ICG, or BSP in the extracellular compartment at the indicated concentrations. Data were corrected for uptake in Na^+-free buffer. Results are the mean ± S.D. from three independent experiments.

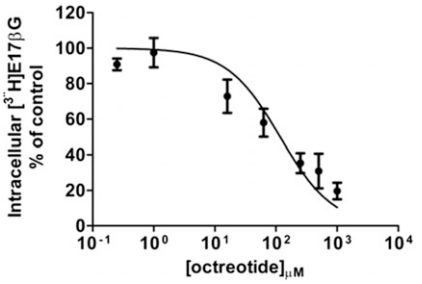

Fig. 4. Effect of octreotide on MRP2 transport activity. MRP2 expressing Sf21 vesicles were incubated for 90 seconds at pH 7.4 with 10 μM [^{3}H]E17βG and in the presence of increasing extravesicular concentrations of octreotide. Transport was measured either in the presence or absence of 5 mM ATP. The specific MRP2-mediated uptake was the difference between the uptake values in the presence and absence of ATP. Results are the mean ± S.D. from three independent experiments.

through the point of origin, indicating unidirectional flux of [^{3}H]E17βG into vesicles (unpublished data). Figure 4 shows the influx of [^{3}H]E17βG at an extracellular concentration of 10 μM in the presence of nonlabeled octreotide in the extracellular compartment at the indicated concentrations. Octreotide inhibited the influx of [^{3}H]E17βG, and the inhibition was concentration dependent. The IC_{50} was computed to be 116.6 ± 1.2 μM (95% CI 74.5–182.4).

Kinetic Analysis of Inhibition of OATP1B1-Mediated Transport by Octreotide. To study the nature of the inhibitory effect of octreotide on [^{3}H]E17βG OATP1B1-mediated transport, influx kinetics of [^{3}H]E17βG were determined in the presence or absence of extracellular octreotide in OATP1B1-CHO cells. Influx of [^{3}H]E17βG as a function of concentration was assessed over 1 minute in the presence or absence of 60 μM extracellular octreotide and was found to be saturable (Fig. 5A). Table 1 summarizes the kinetics based upon the Michaelis-Menten nonlinear regression equation. The [^{3}H]E17βG influx K_m in the presence (K_{mapp}) or absence of octreotide was calculated at 8.8 ± 3.1 and 4.4 ± 1.2 μM, respectively (P = 0.03). The dissociation coefficient (K_i) of octreotide for E17βG was computed to be 33.5 ± 5.5 μM. Thus, the maximal transport capacity (V_{max}) remained unchanged, suggesting fully competitive inhibition of [^{3}H]E17βG influx by nonlabeled octreotide (107.4 ± 8.9 versus 107.0 ± 9.9 pmol/mg of protein/min, P = NS). Figure 5B shows the [^{3}H]E17βG OATP1B1-mediated influx at two different concentrations of substrate as the function of extracellular nonlabeled octreotide at the indicated concentrations (Dixon analysis). It can be seen that the nature of the inhibition was confirmed to be fully competitive, and the K_i value could be estimated to be ~35 μM.

Impact of OATP1B1 on [^{3}H]octreotide Transport. To assess whether octreotide is also a substrate for OATP1B1, the net uptake of 10 μM [^{3}H]octreotide was measured in OATP1B1-CHO cells. Figure 6 shows that the intracellular accumulation of [^{3}H]octreotide over 10- and 30-minute incubation periods was only slightly higher in OATP1B1-CHO cells compared with WT-CHO, suggesting a low transport rate even at a substrate concentration that approached the K_i value. It can be observed that after 30-minute incubation at the extracellular concentration of 10 μM, the intracellular [^{3}H]octreotide was only ~20% higher in OATP1B1-CHO cells compared with that in WT-CHO cells (259.3 ± 32.0 versus 217.1 ± 13.5 pmol/mg of protein, P = NS). However, notably there was substantial background transport/bound level in WT-CHO cells, making further analysis of the kinetics of OATP1B1 mediated transport difficult. This was either due to strong membrane binding and/or nonspecific uptake of [^{3}H]octreotide by an endogenous transport system (Beschiaschvili and Seelig, 1991).

Effect of Extracellular Octreotide on the Efflux of [^{3}H]E17βG Mediated by OATP1B1. To better understand whether octreotide was transported by OATP1B1, its ability to *trans*-stimulate the OATP1B1-mediated efflux of intracellular [^{3}H]E17βG was assessed (Eraly, 2008). Cells were incubated for 30 minutes with 10 μM [^{3}H]E17βG in transport buffer to achieve high intracellular levels. After an extensive washing at 0°C, the cells were resuspended into a large volume of drug-free 37°C transport buffer and the decline in intracellular [^{3}H]E17βG was monitored. In parallel, the efflux rate of [^{3}H]E17βG was monitored in cells resuspended in 37°C transport buffer containing 5 μM BSP (substrate), 5 μM ICG (inhibitor), or 100 μM octreotide. At these concentrations based on the calculated K_i/K_t the carrier molecules in the outward conformation should approach the saturation. It can be seen that the OATP1B1-mediated transport was bidirectional and its cycling rate was maximal when the substrate was present at both sides of the plasma membrane. In fact, the decline of efflux of intracellular [^{3}H]E17βG was enhanced by approximately 2-fold in cells exposed to BSP compared with that in cells incubated in drug-free transport buffer (−0.0050 ± 0.0007 versus −0.0087 ± 0.0017 second^{-1}, P = 0.04) (Fig. 7A). Unlike BSP, ICG reduced the efflux rate of intracellular [^{3}H]E17βG by ~50% (−0.0056 ± 0.0004 versus −0.0038 ± 0.0011 second^{-1}, P = 0.06) (Fig. 7B). Figure 7C shows that octreotide, like BSP, was able to enhance the efflux rate of [^{3}H]E17βG (−0.0053 ± 0.0007 versus −0.0088 ± 0.0015 second^{-1}, P = 0.007), confirming that octreotide was substrate of the OATP1B1.

Discussion

Octreotide, like several other peptides, is rapidly cleared by the liver, and this process is carrier mediated (Gores et al., 1989; Ziegler and Seeberger, 1993; Terasaki et al., 1995; Lamberts et al., 1996). However, it is still unclear which transporters mediate the vectorial transport of octreotide from portal blood into bile. This study establishes that octreotide interacts, to a different extent, with the bilirubin transporters

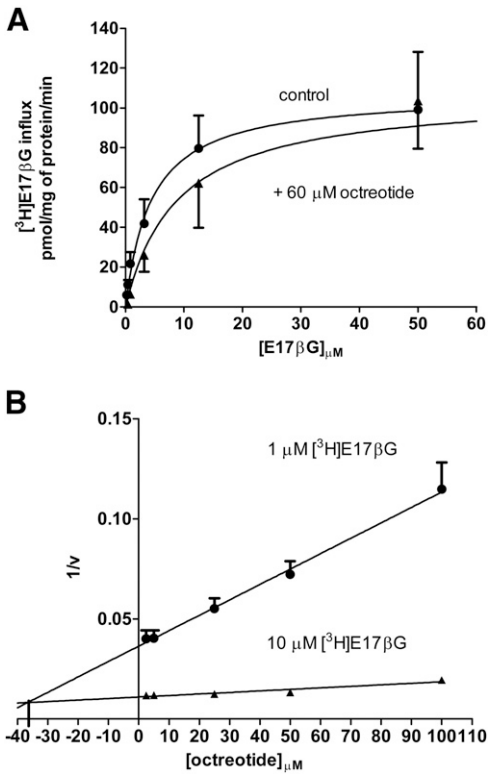

Fig. 5. Kinetic analysis of the inhibition of [^{3}H]E17βG influx by nonlabeled octreotide. Initial uptake of [^{3}H]E17βG was assessed over 1 minute at pH 7.4 in OATP1B1-transfected CHO cells. Data were corrected for uptake in CHO wild-type cells. (A) [^{3}H]E17βG influx as a function of the extracellular concentration of substrate and in the presence or absence of nonlabeled octreotide at an extracellular concentration of 60 μM. Results are the mean ± S.D. from four independent experiments. The line is best-fit to the Michaelis-Menten equation [$V = V_{max}[S]/(K_t + [S])$]. (B) [^{3}H]E17$\beta$G influx at the indicated extracellular concentrations (1 or 10 μM) as a function of increasing extracellular concentrations of nonlabeled octreotide. The line is derived by plotting 1/v against the concentration of octreotide (inhibitor) (Dixon analysis) and represents the mean ± S.D. from three independent experiments.

OATP1B1 and OATP1B3 and, at the canalicular domain, MRP2. Inhibition of this panel of transporters is consistent with their overlapping substrate specificities (Suzuki and Sugiyama, 1999). The data are in line with the observation that patients treated with octreotide can experience hyperbilirubinemia (Arosio et al., 1988; Crook and Steger, 2001; Koren et al., 2013). Bilirubin is taken up into hepatocytes in part by a carrier-mediated process involving OATPs 1B1, 1B3, and 2B1 (Kullak-Ublick et al., 2001; Stieger et al., 2012), is rapidly conjugated to bilirubin glucuronides by uridine diphosphate-glucuronosyl transferase (Erlinger et al., 2014), and is then excreted from hepatocytes into bile canaliculi by the multidrug resistance-associated protein 2 (MRP2) (Kamisako et al., 1999).

The finding that octreotide, formal charge z = +2 at pH 7.4, showed a relatively good affinity (K_i ~35 μM) for OATP1B1 was not surprising and consistent with the ability of OATPs to accommodate not only organic anions but also neutral and cationic molecules (Bossuyt et al., 1996; van Montfoort et al., 2002). Octreotide appears also to be a transport substrate of OATP1B1, although an accurate direct measurement of [^{3}H]octreotide uptake mediated by OATP1B1 was not possible. This was due to a high background signal measured in the nontransfected CHO cells. This effect could not be abolished nor reduced when the experiment was performed in ice, suggesting that the signal was not due to a temperature-dependent process, hence no endogenous carrier was likely to account for such effect. It is possible that this background signal resulted from a substantial membrane-bound component due to lipid-protein electrostatic/hydrophobic interactions (Beschiaschvili and Seelig, 1991). The transport rate of [^{3}H]octreotide uptake mediated by OATP1B1 was slow, even under conditions that approach the influx K_i value, suggesting the octreotide is a better inhibitor than transport substrate of OATP1B1. A marked difference in substrate and inhibitor specificity is consistent with previous studies that have shown other compounds to be strong inhibitors but poor substrates of OATPs (Gui et al., 2008; de Graaf et al., 2011). From a kinetic point of view, a compound that is capable of inhibiting the transport of another substrate without itself being transported as well should be characterized by a relatively good dissociation constant at the extracellular level (K_i/K_{on}) but poor catalytic activity (V_{max}/K_t) because an additional event in the carrier cycle other than the extracellular binding is sufficiently great compared with the K_i/K_{on} to impair the entire cycle of the carrier (Eraly, 2008). Surprisingly octreotide in the extracellular compartment was able to *trans*-stimulate the efflux of intracellular [^{3}H]E17βG (heteroexchange) as efficiently as BSP, a known substrate of OATP1B1 (Kullak-Ublick et al., 2001). Because the *trans*-stimulation usually enhances the cycling rate of the carrier without affecting the binding, it can be assumed that the octreotide transport itself was stimulated as well by the presence of [^{3}H]E17βG at the intracellular level (Goldman, 1971; Visentin et al., 2012, 2015). This suggests that the outward-to-inward translocation rate of the octreotide-OATP1B1 complex was slow in a zero-trans condition (no substrate at the opposite side of the plasma membrane) resulting in a slow uptake rate, but presumably this was overcome in the infinite-trans condition (another substrate in excess at the opposite side of the membrane) when the cycling rate was maximized by the

TABLE 1.

Comparison of [^{3}H]E17βG influx kinetic parameters in the presence or absence of nonlabeled octreotide

The inhibition constant (K_i) was determined from the formula: $K_{mapp} = K_m(1+[I]/K_i)$, where K_{mapp} and K_m are the affinity constants in the presence or absence of octreotide, respectively; [I] represents the extracellular concentration of octreotide. Data are the mean ± S.D. from four independent experiments.

	K_m	V_{max}	V_{max}/K_m	K_i
	μM	*pmol/mg per minute*		μM
Control	4.4 ± 1.2	107.4 ± 8.9	23.9 ± 9.7	—
+60 μM octreotide	8.8. ± 3.1*	107.0 ± 9.9	11.4 ± 5.7§	33.5 ± 5.5

*P value 0.03, for the difference between K_m values.
§P value 0.01, for the difference between V_{max}/K_m values.
—: there is no inhibitor hence no K_i can be measured.

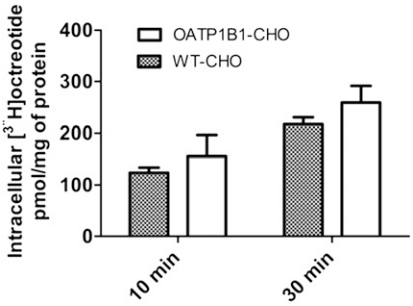

Fig. 6. Uptake of [^{3}H]octreotide mediated by OATP1B1. Uptake of 10 μM [^{3}H]octreotide was assessed at the indicated time at pH 7.4 in OATP1B1-transfected CHO cells and in WT-CHO cells. Results are the mean ± S.D. from three independent experiments.

presence of substrates at both sides of the plasma membrane. This was not the case for ICG, which was found to be a strong OATP1B1 inhibitor, but no transport could be measured (de Graaf et al., 2011). ICG at the extracellular compartment did not stimulate but rather inhibited the efflux rate of intracellular [^{3}H]E17βG. The two possible explanations for this effect are that: 1) ICG locked the carrier in the outward conformation or 2) the ICG-OATP1B1 complex did not dissociate at the intracellular level (K_{off}), depleting the unloaded carrier fraction accessible for [^{3}H]E17βG export. These findings confirm that the inhibition potency of a compound does not necessarily imply that it is a transport substrate and suggest that this *trans*-setting may represent a rapid approach to discriminate between a substrate and a pure inhibitor without direct assessment of the uptake of the radiolabeled or fluorescent equivalent (Apiwattanakul et al., 1999; Bakhiya et al., 2003). In fact, unlike in the *cis*-setting in which they confer similar effects, substrate and inhibitor act differently in the *trans*-setting, allowing rapid discrimination. This approach, together with the classic *cis*-inhibition assay, might allow the rapid screening of potential substrates of the OATPs.

Octreotide showed a relatively strong inhibitory effect on OATP1B1, whereas MRP2 was only weakly inhibited. This finding is consistent with a previous study showing that in Eisai hyperbilirubinemic rats (EHBR), that lack MRP2 expression, the bile-to-liver concentration ratio of octreotide was comparable to that in normal Sprague-Dawley rats, suggesting that MRP2 at best plays a marginal role in the canalicular efflux of octreotide in rodents (Yamada et al., 1996). Nevertheless, the same study found that the overall hepatic excretion of octreotide was reduced in EHBR rats and that this was due solely to a reduced liver-to-plasma concentration ratio. The hypothesis was that other MRP2 substrates accumulated in the liver of EHBR rats and were fluxed back into the blood stream where they competed with octreotide at the sinusoidal level. Whether the sinusoidal transport of octreotide in EHBR rats could be mediated by OATPs was not demonstrated but is a reasonable assumption, considering that OATPs and MRP2 share a variety of substrates (Suzuki and Sugiyama, 1999). For instance, bilirubin glucuronide, a substrate of OATPs and MRP2, is ~50 times more concentrated in the plasma of EHBR than normal rats (49 versus 1 μM) and may inhibit the uptake of octreotide into the hepatocytes (Sathirakul et al., 1993; Yamada et al., 1997).

The current work establishes that octreotide may reduce Na^+-independent bile acid uptake mainly by inhibiting OATP1B1-mediated transport. No interaction was found with NTCP, the Na^+-dependent bile acid transport system, in accordance with the observation that cationic compounds poorly interfere with sodium-dependent taurocholate uptake into hepatocytes (Hardison et al., 1984). This finding contrasts with the previously reported marked reduction of [^{14}C]octreotide uptake in primary rat hepatocytes in the absence of Na^+ or in the presence of ouabain, which collapses the Na^+ gradient across the cell membrane (Terasaki et al., 1995). One possible explanation for this discrepancy is that replacement of Na^+ or the collapse of its gradient alters the membrane potential and/or pH-gradients and results in nonspecific effects. However in the same study, the uptake of [^{14}C]octreotide was more strongly inhibited by BSP (IC_{50} ~10 μM) than by taurocholate (IC_{50} ~100 μM), a pattern that suggests an OATP rather than an NTCP inhibition (Hagenbuch and Meier, 1994; Shitara et al., 2009).

This study showed a clear inhibitory effect of octreotide on the hepatic bilirubin carriers with a pronounced effect on OATP1B1 transport activity. This finding could be relevant for the prediction of potential drug-drug interactions. Octreotide, when administered concomitantly, has been shown to increase the bioavailability of the OATP1B1 substrate bromocriptine by 40% (Flogstad et al., 1994; Lu et al., 2006). Additionally, octreotide is rapidly cleared by the liver, suggesting an extensive first-pass elimination if administered orally and requiring much higher doses to achieve similar blood peak concentrations (C_{max} ~ 5 nM) (Tuvia et al., 2012). At such doses octreotide could reach the liver at much higher local concentrations and could thereby modulate the clearance and increase plasma levels of other OATP1B1 substrates.

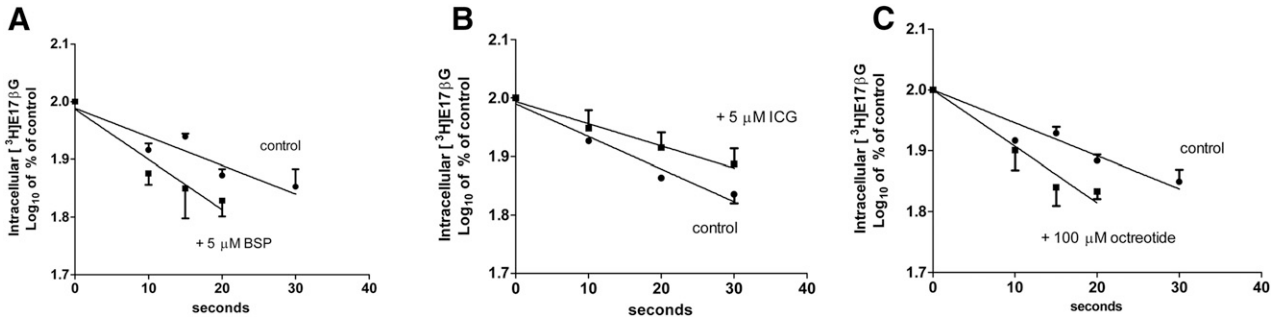

Fig. 7. Analysis of [^{3}H]E17βG efflux mediated by OATP1B1. OATP1B1- and WT-CHO cells were incubated for 30 minutes with 10 μM [^{3}H]E17βG. After intracellular [^{3}H]E17βG was assessed, cells were washed at 0°C and then resuspended either in drug-free buffer or in buffer containing BSP (A), ICG (B), or octreotide (C) at 37°C at the indicated concentrations. The decline in intracellular [^{3}H]E17βG was monitored. The *y* axis is expressed as logarithmic (base 10) scale. Values are the mean ± S.D. from at least three independent experiments.

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Authorship Contributions

Participated in research design: Visentin, Stieger, Merz, Kullak-Ublick.

Conducted experiments: Visentin.

Performed data analysis: Visentin.

Wrote or contributed to the writing of the manuscript: Visentin, Stieger, Merz, Kullak-Ublick.

References

Apiwattanakul N, Sekine T, Chairoungdua A, Kanai Y, Nakajima N, Sophasan S, and Endou H (1999) Transport properties of nonsteroidal anti-inflammatory drugs by organic anion transporter 1 expressed in Xenopus laevis oocytes. *Mol Pharmacol* **55**:847–854.

Arosio M, Bazzoni N, Ambrosi B, and Faglia G (1988) Acute hepatitis after treatment of acromegaly with octreotide. *Lancet* **2**:1498.

Bakhiya A, Bahn A, Burckhardt G, and Wolff N (2003) Human organic anion transporter 3 (hOAT3) can operate as an exchanger and mediate secretory urate flux. *Cell Physiol Biochem* **13**:249–256.

Bauer W, Briner U, Doepfner W, Haller R, Huguenin R, Marbach P, Petcher TJ, and Pless (1982) SMS 201-995: a very potent and selective octapeptide analogue of somatostatin with prolonged action. *Life Sci* **31**:1133–1140.

Ben-Tal N, Honig B, Miller C, and McLaughlin S (1997) Electrostatic binding of proteins to membranes. Theoretical predictions and experimental results with charybdotoxin and phospholipid vesicles. *Biophys J* **73**:1717–1727.

Berelowitz M, Kronheim S, Pimstone B, and Shapiro B (1978) Somatostatin-like immunoreactivity in rat blood. Characterization, regional differences, and responses to oral and intravenous glucose. *J Clin Invest* **61**:1410–1414.

Beschiaschvili G and Seelig J (1991) Peptide binding to lipid membranes. Spectroscopic studies on the insertion of a cyclic somatostatin analog into phospholipid bilayers. *Biochim Biophys Acta* **1061**:78–84.

Bossuyt X, Müller M, and Meier PJ (1996) Multispecific amphipathic substrate transport by an organic anion transporter of human liver. *J Hepatol* **25**:733–738.

Crook MA and Steger A (2001) Abnormal liver function tests in a patient fed with total parenteral nutrition and treated with octreotide. *Nutrition* **17**:152–154.

de Graaf W, Häusler S, Heger M, van Ginhoven TM, van Cappellen G, Bennink RJ, Kullak-Ublick GA, Hesselmann R, van Gulik TM, and Stieger B (2011) Transporters involved in the hepatic uptake of (99m)Tc-mebrofenin and indocyanine green. *J Hepatol* **54**:738–745.

de Waart DR, Häusler S, Vlaming ML, Kunne C, Hänggi E, Gruss HJ, Oude Elferink RP, and Stieger B (2010) Hepatic transport mechanisms of cholyl-L-lysyl-fluorescein. *J Pharmacol Exp Ther* **334**:78–86.

de Waart DR, Paulusma CC, Kunne C, and Oude Elferink RP (2006) Multidrug resistance associated protein 2 mediates transport of prostaglandin E2. *Liver Int* **26**:362–368.

Eraly SA (2008) Implications of the alternating access model for organic anion transporter kinetics. *J Membr Biol* **226**:35–42.

Erlinger S, Arias IM, and Dhumeaux D (2014) Inherited disorders of bilirubin transport and conjugation: new insights into molecular mechanisms and consequences. *Gastroenterology* **146**:1625–1638.

Fløgstad AK, Halse J, Grass P, Abisch E, Djøseland O, Kutz K, Bodd E, and Jervell J (1994) A comparison of octreotide, bromocriptine, or a combination of both drugs in acromegaly. *J Clin Endocrinol Metab* **79**:461–465.

Gerloff T, Stieger B, Hagenbuch B, Madon J, Landmann L, Roth J, Hofmann AF, and Meier PJ (1998) The sister of P-glycoprotein represents the canalicular bile salt export pump of mammalian liver. *J Biol Chem* **273**:10046–10050.

Goldman ID (1971) A model system for the study of heteroexchange diffusion: methotrexate-folate interactions in L1210 leukemia and Ehrlich ascites tumor cells. *Biochim Biophys Acta* **233**:624–634.

Gores GJ, Kost LJ, Miller LJ, and LaRusso NF (1989) Processing of cholecystokinin by isolated liver cells. *Am J Physiol* **257**:G242–G248.

Gui C, Miao Y, Thompson L, Wahlgren B, Mock M, Stieger B, and Hagenbuch B (2008) Effect of pregnane X receptor ligands on transport mediated by human OATP1B1 and OATP1B3. *Eur J Pharmacol* **584**:57–65.

Gutmann H, Miller DS, Droulle A, Drewe J, Fahr A, and Fricker G (2000) P-glycoprotein- and mrp2-mediated octreotide transport in renal proximal tubule. *Br J Pharmacol* **129**:251–256.

Guyot C, Hofstetter L, and Stieger B (2014) Differential effects of membrane cholesterol content on the transport activity of multidrug resistance-associated protein 2 (ABCC2) and of the bile salt export pump (ABCB11). *Mol Pharmacol* **85**:909–920.

Hagenbuch B and Meier PJ (1994) Molecular cloning, chromosomal localization, and functional characterization of a human liver Na+/bile acid cotransporter. *J Clin Invest* **93**:1326–1331.

Hardison WG, Bellentani S, Heasley V, and Shellhamer D (1984) Specificity of an Na+ -dependent taurocholate transport site in isolated rat hepatocytes. *Am J Physiol* **246**:G477–G483.

Hussaini SH, Murphy GM, Kennedy C, Besser GM, Wass JA, and Dowling RH (1994) The role of bile composition and physical chemistry in the pathogenesis of octreotide-associated gallbladder stones. *Gastroenterology* **107**:1503–1513.

Kamisako T, Leier I, Cui Y, König J, Buchholz U, Hummel-Eisenbeiss J, and Keppler D (1999) Transport of monoglucuronosyl and bisglucuronosyl bilirubin by recombinant human and rat multidrug resistance protein 2. *Hepatology* **30**: 485–490.

Kim NS and Lee GM (2000-2001) Overexpression of bcl-2 inhibits sodium butyrate-induced apoptosis in Chinese hamster ovary cells resulting in enhanced humanized antibody production. *Biotechnol Bioeng* **71**:184–193.

Koren I, Riskin A, Barthlen W, and Gillis D (2013) Hepatitis in an infant treated with octreotide for congenital hyperinsulinism. *J Pediatr Endocrinol Metab* **26**:183–185.

Kullak-Ublick GA, Ismair MG, Stieger B, Landmann L, Huber R, Pizzagalli F, Fattinger K, Meier PJ, and Hagenbuch B (2001) Organic anion-transporting polypeptide B (OATP-B) and its functional comparison with three other OATPs of human liver. *Gastroenterology* **120**:525–533.

Lamberts SW, van der Lely AJ, de Herder WW, and Hofland LJ (1996) Octreotide. *N Engl J Med* **334**:246–254.

Lemaire M, Azria M, Dannecker R, Marbach P, Schweitzer A, and Maurer G (1989) Disposition of sandostatin, a new synthetic somatostatin analogue, in rats. *Drug Metab Dispos* **17**:699–703.

Lu WJ, Huang K, Lai ML, and Huang JD (2006) Erythromycin alters the pharmacokinetics of bromocriptine by inhibition of organic anion transporting polypeptide C-mediated uptake. *Clin Pharmacol Ther* **80**:421–422.

Patel YC and Wheatley T (1983) In vivo and in vitro plasma disappearance and metabolism of somatostatin-28 and somatostatin-14 in the rat. *Endocrinology* **112**:220–225.

Pereira SP, Hussaini SH, Murphy GM, Wass JA, and Dowling RH (2001) Octreotide increases the proportions of arachidonic acid-rich phospholipids in gall-bladder bile. *Aliment Pharmacol Ther* **15**:1435–1443.

Radetti G, Gentili L, Paganini C, and Messner H (2000) Cholelithiasis in a newborn following treatment with the somatostatin analogue octreotide. *Eur J Pediatr* **159**:550.

Sathirakul K, Suzuki H, Yasuda K, Hanano M, Tagaya O, Horie T, and Sugiyama Y (1993) Kinetic analysis of hepatobiliary transport of organic anions in Eisai hyperbilirubinemic mutant rats. *J Pharmacol Exp Ther* **265**:1301–1312.

Schlatter E, Mönnich V, Cetinkaya I, Mehrens T, Ciarimboli G, Hirsch JR, Popp C, and Koepsell H (2002) The organic cation transporters rOCT1 and hOCT2 are inhibited by cGMP. *J Membr Biol* **189**:237–244.

Schroeder A, Eckhardt U, Stieger B, Tynes R, Schteingart CD, Hofmann AF, Meier PJ, and Hagenbuch B (1998) Substrate specificity of the rat liver Na(+)-bile salt cotransporter in Xenopus laevis oocytes and in CHO cells. *Am J Physiol* **274**: G370–G375.

Shitara Y, Nagamatsu Y, Wada S, Sugiyama Y, and Horie T (2009) Long-lasting inhibition of the transporter-mediated hepatic uptake of sulfobromophthalein by cyclosporin a in rats. *Drug Metab Dispos* **37**:1172–1178.

Stieger B, Heger M, de Graaf W, Paumgartner G, and van Gulik T (2012) The emerging role of transport systems in liver function tests. *Eur J Pharmacol* **675**: 1–5.

Suzuki H and Sugiyama Y (1999) Transporters for bile acids and organic anions. *Pharm Biotechnol* **12**:387–439.

Tamai I, Nozawa T, Koshida M, Nezu J, Sai Y, and Tsuji A (2001) Functional characterization of human organic anion transporting polypeptide B (OATP-B) in comparison with liver-specific OATP-C. *Pharm Res* **18**:1262–1269.

Terasaki T, Mizuguchi H, Itoho C, Tamai I, Lemaire M, and Tsuji A (1995) Hepatic uptake of octreotide, a long-acting somatostatin analogue, via a bile acid transport system. *Pharm Res* **12**:12–17.

Theodoropoulou M and Stalla GK (2013) Somatostatin receptors: from signaling to clinical practice. *Front Neuroendocrinol* **34**:228–252.

Thévenod F, Ciarimboli G, Leistner M, Wolff NA, Lee WK, Schatz I, Keller T, Al-Monajjed R, Gorboulev V, and Koepsell H (2013) Substrate- and cell contact-dependent inhibitor affinity of human organic cation transporter 2: studies with two classical organic cation substrates and the novel substrate cd2+. *Mol Pharm* **10**:3045–3056.

Toumpanakis C and Caplin ME (2013) Update on the role of somatostatin analogs for the treatment of patients with gastroenteropancreatic neuroendocrine tumors. *Semin Oncol* **40**:56–68.

Treiber A, Schneiter R, Häusler S, and Stieger B (2007) Bosentan is a substrate of human OATP1B1 and OATP1B3: inhibition of hepatic uptake as the common mechanism of its interactions with cyclosporin A, rifampicin, and sildenafil. *Drug Metab Dispos* **35**:1400–1407.

Tuvia S, Atsmon J, Teichman SL, Katz S, Salama P, Pelled D, Landau I, Karmeli I, Bidlingmaier M, and Strasburger CJ, et al. (2012) Oral octreotide absorption in human subjects: comparable pharmacokinetics to parenteral octreotide and effective growth hormone suppression. *J Clin Endocrinol Metab* **97**:2362–2369.

van Montfoort JE, Schmid TE, Adler ID, Meier PJ, and Hagenbuch B (2002) Functional characterization of the mouse organic-anion-transporting polypeptide 2. *Biochim Biophys Acta* **1564**:183–188.

Visentin M, Unal ES, Najmi M, Fiser A, Zhao R, and Goldman ID (2015) Identification of Tyr residues that enhance folate substrate binding and constrain oscillation of the proton-coupled folate transporter (PCFT-SLC46A1). *Am J Physiol Cell Physiol* **308**:C631–C641.

Visentin M, Zhao R, and Goldman ID (2012) Augmentation of reduced folate carrier-mediated folate/antifolate transport through an antiport mechanism with 5-aminoimidazole-4-carboxamide riboside monophosphate. *Mol Pharmacol* **82**: 209–216.

Yamada T, Niinuma K, Lemaire M, Terasaki T, and Sugiyama Y (1996) Mechanism of the tissue distribution and biliary excretion of the cyclic peptide octreotide. *J Pharmacol Exp Ther* **279**:1357–1364.

Yamada T, Niinuma K, Lemaire M, Terasaki T, and Sugiyama Y (1997) Carrier-mediated hepatic uptake of the cationic cyclopeptide, octreotide, in rats. Comparison between in vivo and in vitro. *Drug Metab Dispos* **25**:536–543.

Ziegler K and Seeberger A (1993) Hepatocellular uptake of peptides–I. Carrier-mediated uptake of hydrophilic linear peptides with renin inhibitory activity into isolated rat liver cells. *Biochem Pharmacol* **45**:909–916.

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